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Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae: an experimental approach based on *Artemia* enrichment

J.F. Rees[a,b,1], K. Curé[b], S. Piyatiratitivorakul[a], P. Sorgeloos[b,*], P. Menasveta[a]

[a]*Sichang Marine Science Research and Training Station, Chulalongkorn University, Sichang, 20120 Chonburi, Thailand*

[b]*Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Rozier 44, 9000 Ghent, Belgium*

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Abstract

Penaeus monodon postlarvae (PL-5 to PL-15) were fed 5 different diets consisting of enriched *Artemia* nauplii with a specific *n*-3 HUFA content. Although the postlarvae grew well on an *Artemia* diet with low HUFA-content, the ability of PL-10 to endure osmotic stress remained low. Feeding *Artemia* enriched with medium levels of *n*-3 HUFA (Σ *n*-3 HUFA = 12.55 mg/g DW) for 5 days considerably enhanced the resistance of PL-10 to osmotic stress and their survival recorded 5 days later. However, very high dietary levels of *n*-3 HUFA (31.2 mg/g DW) did not have any growth-promoting effect, thus suggesting that an excessive supply of *n*-3 may not be beneficial to the shrimp. Our data demonstrate that subjecting postlarvae to reduced salinities for 2 h provides a simple and rapid test for assaying the physiological condition of postlarval shrimp.

1. Introduction

Nutrition research in the 1980's demonstrated the important role of highly unsaturated fatty acids (HUFA) in the metabolism of marine organisms (Watanabe et al., 1983; Sorgeloos and Léger, 1992). Among HUFA, the derivatives of linolenic acid (*n*-3 series) deserved particular attention because of their role in

*Corresponding author. Fax +32-9-2644193.

[1]Present address: Laboratory of Animal Physiology, Catholic University of Louvain, Croix du Sud 5, 1348 Louvain-la-Neuve, Belgium.

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the synthesis of eicosanoid hormones and in cellular metabolism together with the poor ability of marine organisms to synthesize these fatty acids (Kanazawa et al., 1979a).

Marine crustaceans are no exception and qualitative *n*-3 HUFA-requirements have been reported for crabs (Levine and Sulkin, 1984) and shrimp (Kanazawa et al., 1979a, b; Martin, 1980; Read, 1981; Petriella et al., 1984; Léger et al., 1985, 1987; Léger, 1989; Abelin, 1991; Catacutan, 1991).

Kanazawa and collaborators (1979a,b) have reported the importance of eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) acids for *Penaeus japonicus*. Although this species might be able to elongate and desaturate linolenic acid to *n*-3 HUFA, this ability is very limited and cannot satisfy the quantitative needs of the shrimp which must be provided with a sufficient dietary *n*-3 HUFA supply (Kanazawa et al., 1979a).

Despite the widely recognized importance of these dietary components, very little is known about the quantitative *n*-3 HUFA requirements of penaeids, the most extensively cultured crustaceans. Kanazawa (in D'Abramo, 1991) suggested that a dietary provision of 1% *n*-3 HUFA could be considered a minimal value for postlarval penaeids. However, differences in the feeding habits among penaeid species, i.e. from omnivorous to carnivorous, render any generalization hazardous. Therefore, quantitative HUFA requirements of each species should be further investigated.

This work analyzed the *n*-3 HUFA requirement of early postlarval stages of the giant tiger shrimp, *P. monodon*, fed *Artemia* nauplii in which the fatty acid content was manipulated through bioencapsulation with different concentrations of a HUFA-rich oil emulsion.

2. Material and methods

Experimental design

Four-day-old *Penaeus monodon* postlarvae (PL4) were purchased from a commercial hatchery in Chonburi province, Thailand. They were stocked in 15 cylindrical tanks filled with 150 litres filtered (1 μm) and chlorine-disinfected (5 ppm for 24 h) seawater (30 ppt). The stocking density was 20 postlarvae/l.

Postlarvae (PL-5) were reared on 5 different diets (with 3 replicates) all based on live *Artemia* nauplii (Great Salt Lake, Utah, USA). Newly-hatched *Artemia* nauplii were consequently enriched for 12 h with 100 ppm dry baker's yeast (Instant yeast, Bruggeman, Belgium) and 5 concentrations (0, 100, 200, 300 and 400 ppm) of a HUFA-rich oil emulsion (SELCO®, Artemia Systems NV/SA, Belgium) following the procedure of Léger et al. (1987). Baker's yeast was added to prevent starvation of control nauplii, which is known to reduce their lipid content (Benijts et al., 1976). A comparison of the fatty acid content of baker's yeast-fed and newly-hatched nauplii indicated that feeding baker's yeast allowed the maintenance of a stable HUFA-content in the live food. The nauplii density during enrichment was 150/ml. Enriched nauplii were rinsed with seawater and stored

in slightly-aerated cold seawater (2–10°C) to reduce the consumption of HUFA by *Artemia* (Léger et al., 1983). New batches of enriched nauplii were produced daily.

An *ad libitum* feeding regime was applied to all tanks throughout the experiment and live food density was adjusted 4 times a day. Daily water exchange rate was 70%. Temperature ranged from 27 to 29°C.

Due to the high mortalities that occurred in some tanks, the rearing experiment was terminated on day 10.

In an attempt to evaluate the physiological condition of the postlarvae, we measured their resistance to osmotic shock following the method of Tackaert et al. (1989). On days 0, 5, and 10, ten postlarvae were randomly sampled from each tank and transferred into 1-liter beakers filled with fresh water or diluted seawater (5 and 10 ppt). Their survival was monitored at 5-min intervals over a 2-h period. Postlarvae not reacting to gentle mechanical stimulation with a soft paintbrush were considered dead. Non-reacting animals were not removed from the beakers until the end of the test. This procedure allowed some recovery of apparently "dead" individuals and, therefore, it reduced the probability of untimely death diagnostics.

Cumulative mortality index (CMI) was calculated by summing mortality counts measured at each time interval over the 2-h period as follows:

$$CMI = D5 + D10 + \ldots + D120$$

where DX is the number of dead postlarvae at time X in min. The higher the CMI value, the lower the resistance to the salinity shock.

Fatty acid analysis

At the end of the feeding experiment, 15-day-old postlarvae were harvested, freeze-dried and their fatty acid composition was analyzed by capillary gas chromatography following the standard ICES technique (Artemia Reference Center, 1993). The results are expressed as area percent fatty acids methyl esters (FAME) and mg FAME·g^{-1} dry weight (DW). The fatty acid composition of *Artemia* samples taken during the course of the experiment was also analyzed.

Data analysis

Standard length of the postlarvae (the distance from the rostrum's base to the tip of the telson) was measured with a calibrated curvimeter on 100 (PL-10) or 150 (PL-15) shrimp. At the end of the feeding test, remaining postlarvae were counted and survival was calculated after correction for the amount of shrimp that was sampled from each tank. The average individual dry weight (DW) was calculated on triplicate samples of 50 PL-15 dried for 24 h at 60°C. The total production was expressed as a percentage of the initial biomass stocked in individual tanks (485 mg DW/tank). Survival and standard length values were subjected to $\sqrt{\text{arcsin}}$ and log transformation, respectively, prior to statistical analysis.

Data were processed for significant differences among treatments using the

Table 1
Fatty acid composition of *Artemia* metanauplii enriched for 12 h with increasing concentrations of a *n*-3 HUFA-rich emulsion (SELCO)

Fatty acid	*Artemia* enrichment											
	SELCO		Control		100 ppm		200 ppm		300 ppm		400 ppm	
	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g
14:0	6.50	31.70	0.75	0.95	0.85	1.00	1.25	1.75	1.20	2.25	1.15	2.15
14:1	0.20	3.00	0.65	1.20	0.65	0.75	0.60	0.90	0.55	0.90	0.40	0.80
15:0	0.10	1.10	0.15	0.15	0.15	0.15	0.15	0.25	0.15	0.25	0.14	0.20
15:1	0.10	0.80	0.55	0.65	0.50	0.65	0.50	0.70	0.40	0.65	0.30	0.60
16:0	14.90	138.1	11.90	21.10	11.55	14.00	12.30	17.80	11.15	19.75	9.25	17.50
16:1	8.00	100.90	3.50	5.85	3.45	4.30	5.70	8.20	3.60	6.35	3.35	6.30
17:0	0.60	7.40	0.80	1.40	0.70	0.85	0.85	1.25	0.70	1.25	0.60	1.10
16:2	0.30	4.00	0.50	0.65	0.30	0.45	0.50	0.70	0.30	0.55	0.20	0.45
16:3	1.20	15.50	1.05	1.15	0.75	0.95	1.15	1.60	0.75	1.30	0.50	0.95
18:0	3.90	49.20	7.30	12.95	6.70	8.10	6.30	8.95	5.35	9.30	4.25	8.40
18:1	14.10	178.40	23.95	27.50	23.50	28.75	23.80	34.50	22.75	40.40	21.30	40.10
19:0	0.10	0.90	1.05	1.20	0.95	1.15	0.75	1.15	0.75	1.30	0.70	1.35
18:2	1.30	18.90	5.30	5.90	4.90	6.05	4.75	6.65	4.75	8.50	5.05	9.55
19:4	0.30	3.70	0.20	0.25	0.15	0.15	0.05	0.10	0.15	0.30	0.20	0.30
18:3*n*-6	0.20	3.00	0.75	0.85	0.80	0.95	0.45	0.70	0.50	0.90	0.55	0.95
20:0	0.10	1.30	0.20	0.25	0.18	0.19	0.15	0.20	0.14	0.20	0.13	0.20
18:3*n*-3	1.20	14.90	28.05	31.50	26.50	32.70	20.45	30.90	20.55	36.95	21.35	40.30
20:1	1.70	21.2	0.60	0.70	0.90	1.10	1.45	2.20	1.10	1.90	0.95	1.80
18:4	2.90	36.00	4.90	5.50	4.30	5.35	3.45	5.25	3.65	6.60	3.90	7.40
20:3*n*-6	0.10	1.60	0.25	0.25	0.15	0.20	0.10	0.20	0.20	0.30	0.10	0.20
20:4*n*-6	1.00	13.70	2.35	2.60	2.20	2.75	1.90	2.90	2.00	3.65	2.20	4.15
22:1	2.00	25.10	0.03	0.03	0.20	0.25	0.60	0.85	0.60	1.10	0.40	0.80
21:5	0.80	10.70	0.95	1.10	0.95	1.15	0.80	1.25	0.95	1.75	1.10	2.10
20:5*n*-3	16.10	202.70	1.95	3.20	3.50	4.40	5.55	8.50	7.40	13.90	10.35	19.50
22:4*n*-6	0.70	9.10	0.10	0.15	0.10	0.15	0.04	0.05	0.15	0.30	0.25	0.40
24:1	0.30	3.80	nd	nd	nd	nd	0.30	0.40	0.15	0.30	0.05	0.20
22:5*n*-3	2.10	27.10	0.04	0.05	0.14	0.15	0.45	0.80	0.80	1.55	1.15	2.20
22:6*n*-3	10.90	137.3	0.30	0.35	0.60	0.75	2.10	3.25	3.70	8.40	5.10	9.50
Σ *n*-3 HUFA	29.10	367.10	2.29	2.65	4.25	5.30	8.10	12.55	11.80	22.35	16.60	31.20
Σ *n*-6 HUFA	1.80	24.40	2.70	3.00	2.45	3.10	2.04	3.15	2.35	4.75	2.65	4.75
n-3/*n*-6	16.17		0.85		1.71		3.84		4.85		6.26	
Σ HUFA	31.70	402.20	5.94	6.95	7.65	9.10	10.90	16.60	15.10	28.35	20.75	38.05
Lipid content (%)	100		17.26		19.43		21.44		23.86		26.58	
20:5*n*-3/ 22:6*n*-3	1.48		7.25		7.00		2.80		2.17		2.03	

The composition of SELCO is presented in the first column. Instant baker's yeast (100 ppm) was added to all enrichment solutions. Fatty acid content is expressed as area percent FAME and mg FAME/g dry weight. Each value is a mean of two replicate samples.
nd = not detected.

Table 2
Fatty acid composition of *P. monodon* postlarvae fed *Artemia* enriched with increasing concentrations of a *n*-3 HUFA-rich emulsion

Fatty acid	*Artemia* enrichment									
	Control		100 ppm		200 ppm		300 ppm		400 ppm	
	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g
14:0	0.50	0.35	0.50	0.35	0.53	0.37	0.53	0.37	0.47	0.33
14:1	0.30	0.20	0.35	0.20	0.10	0.07	0.37	0.20	0.37	0.27
15:0	0.20	0.14	0.25	0.15	0.23	0.17	0.20	0.09	0.20	0.10
15:1	0.90	0.40	0.80	0.50	0.70	0.47	0.43	0.27	0.50	0.37
14:2	nd	nd	nd	nd	nd	nd	0.07	0.03	0.40	0.30
16:0	14.63	9.05	13.05	7.90	13.33	10.40	13.23	7.93	13.23	9.90
16:1	2.00	1.25	1.80	1.05	1.90	1.50	2.20	1.37	2.17	1.67
17:0	1.07	0.70	0.95	0.60	0.60	0.47	0.93	0.57	1.03	0.80
16:2	0.17	0.08	1.10	0.60	0.60	0.37	0.03	0.02	0.77	0.57
16:3	2.47	1.50	0.85	0.50	1.00	0.93	1.87	1.10	4.07	3.03
18:0	11.07	6.95	9.05	5.50	8.53	6.53	8.03	4.73	8.10	6.07
18:1	23.87	14.75	22.50	13.55	22.03	17.03	22.57	13.50	22.53	16.87
19:0	0.47	0.30	0.35	0.25	0.30	0.23	0.37	0.20	0.33	0.27
18:2	4.90	2.85	4.90	2.95	5.57	4.13	5.00	3.07	4.67	3.47
19:4	0.03	0.03	nd	nd	0.20	0.20	0.20	0.10	0.40	0.30
18:3*n*-6	0.27	0.20	0.25	0.20	0.13	0.10	0.20	0.12	0.17	0.10
20:0	0.33	0.20	nd	nd	0.07	0.10	0.20	0.10	0.20	0.13
18:3*n*-3	17.73	11.30	14.20	8.55	14.43	11.27	14.47	8.73	12.63	9.47
20:1	0.80	0.45	0.60	0.40	0.80	0.67	0.67	0.40	1.00	0.77
18:4	1.13	0.70	0.70	0.45	0.77	0.63	0.87	0.53	0.70	0.53
20:3*n*-6	0.17	0.09	nd	nd	0.10	0.10	0.23	0.13	0.27	0.17
20:4*n*-6	4.87	3.15	3.50	2.05	3.53	2.93	4.43	2.63	4.33	3.23
22:1	0.07	0.00	0.30	0.20	0.17	0.13	0.20	0.13	0.20	0.10
21:5	0.90	0.55	0.35	0.20	0.70	0.60	0.93	0.53	0.87	0.67
20:5*n*-3	6.40	3.90	8.70	5.20	8.10	6.73	10.20	6.03	10.90	8.13
24:1	nd	nd	nd	nd	0.20	0.07	0.13	0.10	0.27	0.20
22:5*n*-3	0.20	0.15	0.20	0.10	0.80	0.37	0.90	0.53	0.90	0.63
22:6*n*-3	0.90	0.55	1.90	1.15	5.10	2.80	6.00	3.60	6.93	5.17
Σ *n*-3 HUFA	7.50	4.60	10.80	6.45	11.43	9.90	17.10	10.17	18.73	13.93
Σ *n*-6 HUFA	5.03	3.24	3.50	2.05	3.63	3.03	4.67	2.76	4.60	3.40
n-3/*n*-6	1.49		3.08		3.13		3.67		4.07	
Σ HUFA	13.43	8.39	14.65	8.70	15.77	13.53	22.70	13.46	24.20	18.00
20:5*n*-3/ 22:6*n*-3	7.14		5.11		3.39		1.71		1.58	

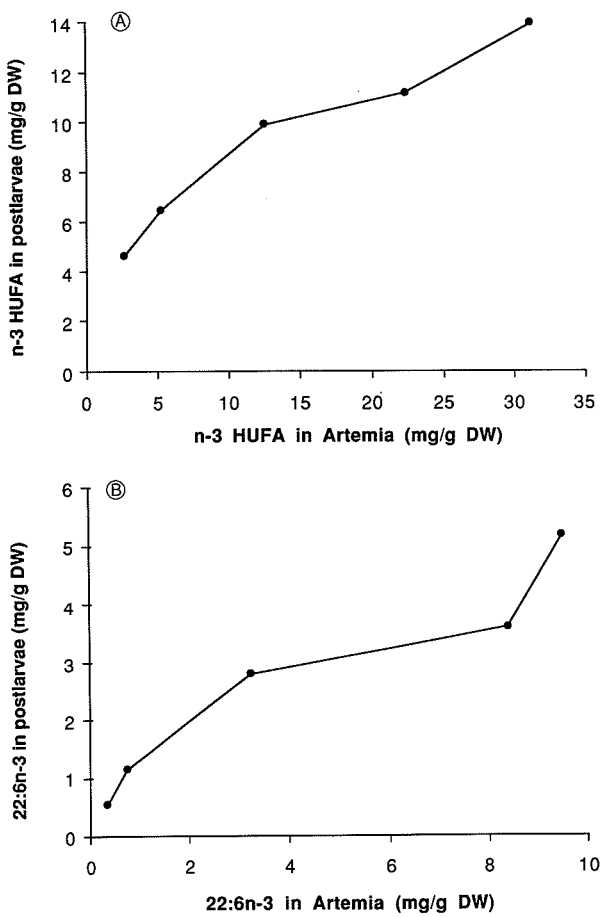
Fatty acid content is expressed as area percent FAME and mg FAME/g dry weight. Each value is a mean of 3 replicate analyses except for the absolute values of the control and the 100-ppm emulsion treatments which are based on two replicates.
nd = not detected.

General Linear Models procedure and Duncan's Multiple Range Comparison analysis (Statistical Analysis System, SAS Institute, USA).

3. Results

Fatty acid composition of Artemia

The fatty acid composition of the *Artemia* diets is summarized in Table 1. The enrichment markedly affected the fatty acid composition of *Artemia*. *n*-3 HUFA showed the highest increment in the nauplii, ranging from 2.65 mg/g DW in controls to 31.2 mg/g DW in the *Artemia* enriched in the 400 ppm emulsion. The *n*-6 HUFA content of *Artemia* was much less affected by the enrichment, ranging from 2.45 mg/g DW in non-enriched nauplii up to 4.75 mg/g DW after enrichment with 400 ppm emulsion. The total lipid content of the *Artemia* increased with the emulsion concentration, i.e. from 17.26% in controls up to 26.57% in *Artemia* enriched in the 400 ppm emulsion. The *n*-3/*n*-6 HUFA ratio ranged from 0.96 in the control to 6.26 in the 400-ppm enrichment treatment while the 20:5*n*-3/22:6*n*-3 ratio decreased from 7.25 to 2.03.



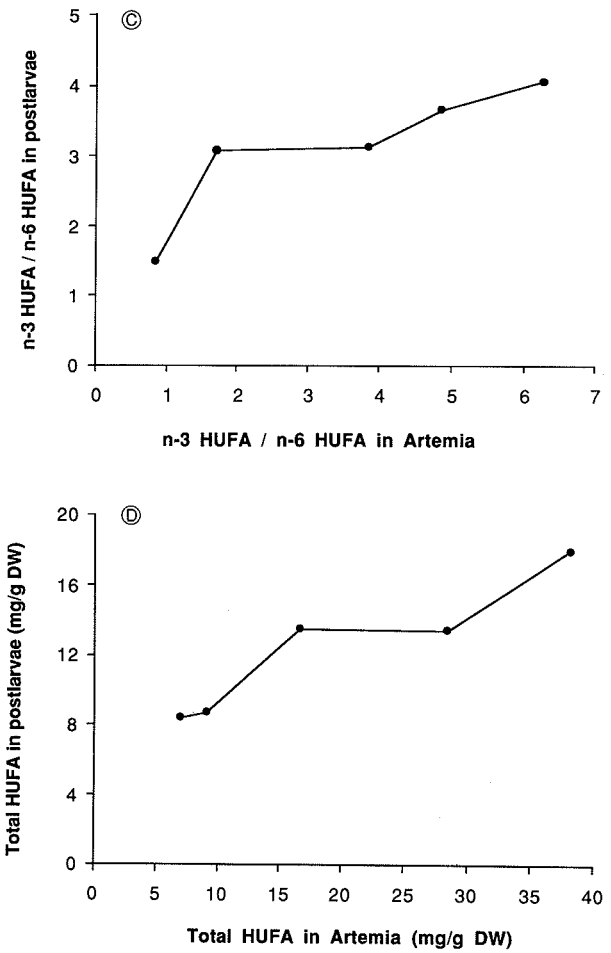


Fig. 1. Relationships between the HUFA composition of *P. monodon* postlarvae and the HUFA content of their *Artemia* diet. (A) Total *n*-3 HUFA; (B) 22:6*n*-3; (C) *n*-3/*n*-6 HUFA; (D) total HUFA. Each value is a mean of two (*Artemia* nauplii) or three (postlarvae) replicate analyses.

Fatty acid composition of postlarvae

The HUFA content of the *Artemia* had a considerable influence on that of the postlarvae (Table 2 and Fig. 1): i.e., the *n*-3 HUFA content of PL-15 increased from 4.60 mg/g DW in the control to 13.93 mg/g DW in postlarvae fed the 400-ppm emulsion enriched *Artemia* (Fig. 1A). 22:6*n*-3 accounted for most of the increase and its concentration ranged from 0.55 to 5.17 mg/g DW in controls and 400-ppm enrichment treatments respectively (Fig. 1B). The 20:5*n*-3/22:6*n*-3 ratio in postlarvae evolved in parallel with that in the *Artemia* diet. However, the 20:5*n*-3/22:6*n*-3 ratio reached lower values in postlarvae than in the *Artemia* diet when the latter contained about 2 times more 20:5*n*-3 than 22:6*n*-3. The *n*-6 HUFA tissue content of the postlarvae remained stable in all treatments. As a

Table 3
Culture performances of *P. monodon* postlarvae fed *Artemia* nauplii enriched with instant baker's yeast (BY) and various amounts of SELCO

Artemia enrichment	Parameters				
	Standard length PL-10 (mm)[1]	Standard length PL-15 (mm)[2]	Individual dry weight PL-15 (mg)[3]	Final survival (%)	Total production (% initial dry weight)[4]
Control	10.30±0.11	11.73±0.11	1.46±0.08	18.84±9.45	59.94±30.48
100 ppm BY	a	a	b	b	c
100 ppm BY+	10.24±0.08	11.75±0.12	1.62±0.09[a]	21.08±13.07	95.82±47.08
100 ppm SELCO	ab	a		b	c
100 ppm BY+	10.13±0.07	11.35±0.10	1.48±0.10	60.15±8.64	456.50±30.33
200 ppm SELCO	ab	b	ab	a	a
100 ppm BY+	9.98±0.09	11.37±0.09	1.41±0.15	62.21±9.21	421.50±14.81
300 ppm SELCO	b	b	b	a	ab
100 ppm BY+	9.40±0.85	11.22±0.09	1.395±0.132	40.84±18.79	239.51±62.00
400 ppm SELCO	c	b	b	ab	bc

Within each column, means (±s.e.m.) with the same superscript are not significantly different ($P<0.05$). Initial dry weight and standard length at PL-5 stage were 0.1618 mg and 6.82 mm, respectively. Values across columns not sharing the same superscript letter are significantly ($P<0.05$) different.
[1]Measured on 100 individuals/treatment.
[2]Measured on 150 individuals/treatment.
[3]From 3 measurements/treatment.
[4]Data from individual tanks ($n=3$).

consequence, the resulting *n*-3/*n*-6 HUFA ratio increased with that in the *Artemia* (Fig. 1C). However, signs of saturation appeared in postlarval tissues when the ratio in *Artemia* reached a value of 3–4. The total HUFA content of postlarvae reflected that of the dietary *n*-3 series (Fig. 1D).

Growth rate

Enrichment of *Artemia* with highly unsaturated fatty acids had no positive effect on the length of the postlarvae. On the contrary, shrimp fed control nauplii achieved the highest growth rate at PL-10 and PL-15 stages (Table 3) whereas those given *Artemia* enriched with either 200, 300 and 400 ppm emulsion were significantly smaller ($P<0.05$) at PL-15. At PL-10, shrimp of the 400-ppm treatment were 10.7% smaller than controls. Five days later, the difference was reduced to 4.3%.

The negative influence of high amounts of dietary *n*-3 HUFA on the growth rate of postlarvae was also reflected in the final dry weight, as the shrimp fed the 300 and 400 ppm diet achieved the poorest weight increments ($P<0.05$). Nevertheless, the individual dry weight of postlarvae was higher in the 100 ppm treatment than in the control ($P<0.05$).

Table 4
Cumulative mortality index (CMI) values measured for *P. monodon* postlarvae (PL-10 and PL-15 stages) reared on *Artemia* enriched with 100 ppm baker's yeast and 5 concentrations of SELCO

Treatment	0 ppt		5 ppt		10 ppt	
	PL-10	PL-15	PL-10	PL-15	PL-10	PL-15
Control (no SELCO)	213.33[a]	203.33	146.33	49.33	90.00[a]	0
100 ppm SELCO	205.67[ab]	204.67	128.33	39.67	43.00[ab]	0
200 ppm SELCO	203.00[b]	206.00	110.00	40.00	6.33[b]	0
300 ppm SELCO	209.67[ab]	204.67	132.00	37.67	28.00[b]	0
400 ppm SELCO	207.33[ab]	194.00	117.00	33.00	12.00[b]	0
	S[1]	NS	NS	NS	S	NS

Postlarvae were transferred from full strength seawater (30 ppt) to freshwater (0 ppt) or diluted seawater (5 and 10 ppt). At PL-5 stage, CMI values at 5 and 10 ppt were 221 and 165, respectively. Each value is a mean of 3 replicates. Within each column, means with the same superscript are not significantly different ($P<0.05$).
[1]$P<0.05$.

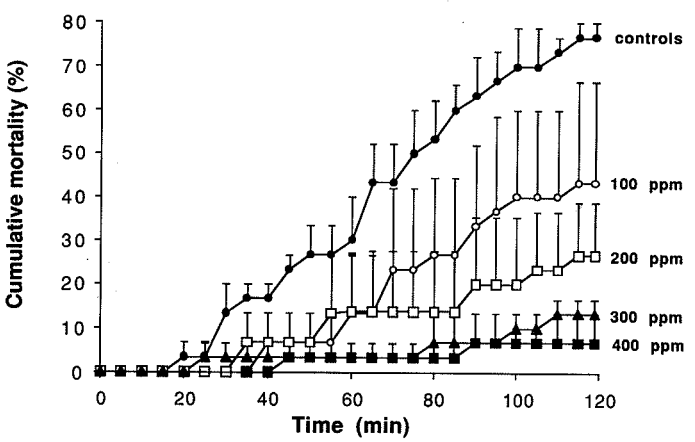


Fig. 2. Time-course of cumulative mortalities recorded when 10-day-old postlarvae fed *Artemia* nauplii enriched with various levels of a HUFA-rich emulsion were transferred from natural seawater (30 ppt) to 10-ppt salinity seawater.

Survival

Survival varied considerably among tanks (8.6–72.1%) and treatments (Table 3). Survival in the control tanks was very low, averaging 18.84±9.45%. Mor-

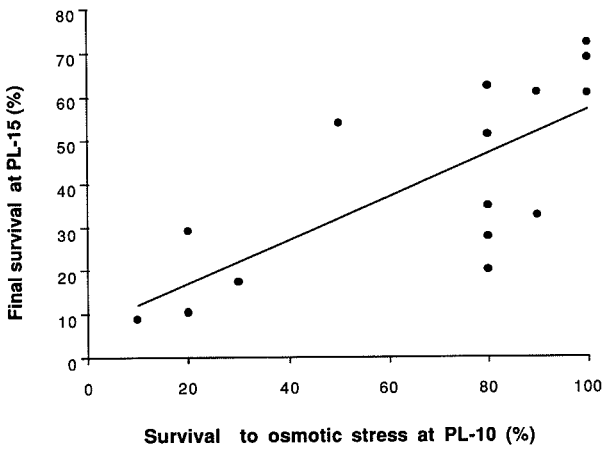


Fig. 3. Relationship ($y=7.01+0.50x$; $r=0.73$) between 2-h survival of PL-10 postlarvae subjected to a 10-ppt salinity shock and final survival measured 5 days later (PL-15) in each tank ($n=15$).

tality remained low in all tanks until day 6 when an increase in the mortality rate occurred in the control and the 100 ppm treatments; similar events were observed on day 8 and 10 in the 400 ppm and 300 ppm tanks, respectively. From these days onwards, the mortality rate remained high in these tanks until the end of the experiment. At no time were there any detectable signs of disease or luminous bacteria present in any tank.

Shrimp fed the 200- and 300-ppm enriched *Artemia* achieved a significantly better survival (>60%) than the control ($P<0.05$); those of the 400-ppm treatment showed an intermediate survival rate, not significantly higher than the control ($P<0.05$).

Total production

The total production varied considerably among treatments. The production in the control tanks averaged only 59.94 ± 30.48% of the biomass stocked 10 days earlier whereas shrimp fed the 200- and 300-ppm enriched *Artemia* gave the best results with a production corresponding to 456.50 ± 30.33 and 421.50 ± 14.81% of the initial biomass ($P<0.05$), respectively. Postlarvae fed *Artemia* enriched with 400 ppm emulsion achieved a significantly lower production than the 200 ppm treatment ($P<0.05$).

Stress resistance

After 5 days of culture, marked differences in the ability of the postlarvae to survive osmotic stress were detected among the 5 treatments. Table 4 summarizes cumulative mortality index (CMI) values recorded for postlarvae challenged with salinities of 0, 5 and 10 ppt.

At 0 ppt, PL-10 fed *Artemia* enriched with 200 ppm emulsion showed a higher resistance (i.e. lower CMI) than the control ($P<0.05$). At 5 ppt, no significant difference was observed among treatments. But, when a milder salinity shock (10 ppt) was applied, the resistance of the groups fed *Artemia* enriched with more

than 100 ppm emulsion was significantly better than in controls ($P<0.05$). After 2 h exposure to 10 ppt, the survival of control shrimp averaged only $23\pm3\%$ whereas it ranged from $56\pm23\%$ to $93\pm7\%$ in the other treatments (Fig. 2). No mortality was recorded when postlarvae were subjected to a 15-ppt stress test.

Although PL-15 shrimp did not survive better than PL-10 when exposed to freshwater, PL-15 achieved a better resistance to the 5-ppt salinity test than PL-10, and shrimp from all treatments resisted a 2-h immersion in 10-ppt diluted seawater. No significant differences in the resistance of the PL-15 shrimp from the 5 treatments could be detected ($P<0.05$).

Remarkably, shrimp of the tanks achieving the best survival to the salinity shock (10-ppt test) at PL-10 also showed the highest final survival 5 days later. The relationship between the two parameters ($r=0.73$; $P=0.0019$) is illustrated in Fig. 3. No significant correlation was found between stress-resistance performance at PL-15 and final survival.

4. Discussion

The above observations suggest that *n*-3 HUFAs are important nutrients for *P. monodon* postlarvae. Effectively, although the growth rate of the postlarvae fed *Artemia* with a high *n*-3 HUFA-content (*n*-3 HUFA $\geqslant 12.55$ mg/g DW) was slightly reduced, their survival was improved to such an extent that the growth reduction appeared negligible. This was reflected by the total biomass production data which clearly demonstrated a beneficial action of *n*-3 HUFA. Nevertheless, our results also suggest that supplying very high *n*-3 HUFA levels (31.2 mg/g DW) to the postlarvae has no positive effect on the survival of the postlarvae.

Previous work by Millamena et al. (1988) and Abelin (1991) has shown that feeding *Artemia* nauplii with increased HUFA content to *P. monodon* postlarvae favored better growth and higher survival rates. While the present study confirms the beneficial action of HUFA on the survival of the postlarvae (Abelin, 1991), it failed to demonstrate any growth-promoting effect of polyunsaturated fatty acids as reported by Millamena et al. (1988). However, the growth reduction which we noted remained moderate as it did not exceed 4.3% of the standard length at PL-15. One could argue that the lower growth in the shrimp fed *Artemia* with the highest HUFA content was perhaps not directly related to HUFA, but could possibly result from differences in the survival rate among treatments: i.e., the much lower survival of postlarvae fed low-HUFA nauplii would reduce the competition for food and space and consequently allow better growth in these tanks. Nevertheless, mortality rate remained very low during the first half of the rearing test in all treatments while significant differences in the growth rate had already become evident. Therefore, the reduced growth rate of the postlarvae may be related to the high HUFA content and/or incorrect balance of specific fatty acids (e.g. *n*-3/*n*-6, 20:5*n*-3/22:6*n*-3, ...) of their diet. In their study, Millamena et al. (1988) compared the culture performance of postlarvae fed *Artemia* enriched with either rice bran (HUFA-deficient) or two algae species containing different

levels of HUFA. They observed that PL-10 reared for 20 days on algae-enriched live food achieved a higher growth than those fed HUFA-poor *Artemia*. Besides the fact that the growth-enhancing effect of enriching *Artemia* with algae might result from an increased supply of algal components other than HUFA, e.g. vitamins, another important difference in our tests is that they were carried out on older postlarval stages (PL-10 to PL-30). As our experiment revealed, the negative effect of high HUFA levels on the growth rate appeared more pronounced on early PL-stages (PL-5 to PL-10) than on later stages. Therefore, it is possible that the apparent contradiction between the present results and those reported by Millamena et al. (1988) simply reflects differences in the metabolism of postlarval stages. If we had carried out the rearing test for another 10 days, we might have come to the same conclusions as Millamena et al. (1988).

Besides the influence of postlarval stage, the effects of HUFA on the culture performance of penaeid postlarvae may also vary from species to species. In *P. vannamei*, Léger et al. (1987) reported improved growth performance when late larval and postlarval stages (M-III to PL-8) were fed HUFA-enriched (600 ppm SELCO, 24 h) *Artemia*, however, survival was not different from the treatment with newly-hatched *Artemia* nauplii. In *P. stylirostris*, both survival and growth performances were improved by feeding mysis and postlarvae (M-II to PL-8) with HUFA-enriched *Artemia* nauplii (Léger et al., 1985). Nevertheless, one must be careful when comparing results obtained for various species as differences may not necessarily reflect specific variations or differences in the requirements of larval and postlarval stages, but could also be related to variations in the nutritional status of the shrimp larvae when they molt into postlarvae. Indeed, Léger et al. (1987) observed that the HUFA content and composition of the diet fed to early larval stages still determines physiological performance in later postlarval stages. Thus, the discrepancies between penaeid species may have partly arisen from interferences in the feeding regimes of previous larval stages. In order to rule out this factor in future comparisons, one should consider applying standardized feeding strategies applicable to all penaeid larvae in larval/postlarval nutrition research.

Although the growth performances of postlarvae were not improved by a feeding regime of HUFA-enriched *Artemia*, it considerably enhanced their ability to withstand stressful environmental conditions. At PL-10, postlarval stress resistance, as measured by their ability to survive osmotic shock (2 h at 10 ppt), increased remarkably. This confirms earlier observations of Tackaert et al. (1989) that the HUFA-composition of the diet can affect the stress resistance of postlarvae. The mechanism underlying this protective effect of HUFA on osmotic resistance in shrimp is not known. Hørstmark et al. (1987) demonstrated that erythrocytes of rats fed HUFA-rich cod liver oil achieved a higher resistance to hypo-osmotic shock, an effect which probably resulted from a higher incorporation of *n*-3 HUFA in cell membranes. If a similar phenomenon also occurs in crustaceans, the better resistance of shrimp fed HUFA-enriched *Artemia* could possibly result from an increased osmotic resistance of their cells, delaying the onset of irreversible damage in some essential tissue. At Agmarina de Panama, (Veracruz,

Panama), R. Chamorro (pers. comm.) observed that the gills in HUFA-enriched postlarvae of *P. stylirostris* and *P. vannamei* of the same stage displayed a more ramified structure. Since the gills are the site of essential osmoregulatory mechanisms in crustaceans, such an increase in the exchange area could result in better resistance to osmotic shocks. Although our results do not exclude the occurrence of such a mechanism, they do not support this phenomenon as the sole reason for the enhanced osmotic resistance of postlarvae. Effectively, one main finding of the present study is that the diet treatments performing best in stress tests at PL-10 were also those with the best survival at the end of the rearing period. As salinity fluctuations were not the cause of the mortality observed in the tanks, this result suggests that the higher resistance to osmotic shock did not result from a specific effect of HUFA on the osmoregulatory properties of the shrimp only, and that it reflects an improvement in the total physiological condition of the postlarvae, presumably induced by the higher dietary *n*-3 HUFA provision. Furthermore, our data also confirm the observation by Tackaert et al. (1989) that HUFA affect postlarval stress resistance more in early stages (PL-5-PL-10) than in later stages (PL-15). Whether this reflects changes in shrimp lipid metabolism or in their osmoregulatory physiology remains an open question.

Besides providing shrimp nutrition researchers with a new test for determining the influence of diet composition on early postlarval stages, this result could be of great help to farmers and hatchery operators. Some commercial hatcheries in South America and South-East Asia already apply similar stress tests to determine the appropriate time for stocking postlarvae (Sorgeloos, 1989). Our results give some scientific support to this empirical practice. Such a test is rapid, easy and inexpensive as it requires no sophisticated equipment. Therefore, we believe its use should be advertised among farmers and hatchery operators as a way to define quality standards and to improve hatchery outputs. Further tests should be carried out to investigate the possibility of applying such a stress test at older postlarval stages.

Improvements in resistance to osmotic stress were already detectable when the HUFA content of the *Artemia* increased from 6.95 to 9.10 mg/g DW, whereas maximal resistance was reached when postlarvae were fed *Artemia* containing 16.60 mg/g DW HUFA (200 ppm emulsion). It can be noted that the maximal production at PL-15 was achieved by postlarvae fed nauplii with the same HUFA content. One might therefore conclude that, under our experimental conditions, the quantitative HUFA requirements might be close to that value. A further examination of the HUFA content of this *Artemia* diet showed that *n*-3 HUFA were by far the most abundant (12.55 mg/g DW or 8.1% of the total fatty acid content) and they represented as much as 76% of the total HUFA content. On the other hand, the *n*-6 HUFA content was only 3.15 mg/g DW or 2.04% of the total fatty acids. When expressed as percent of the total dry weight of the diet, these values were 1.26 and 0.41% for *n*-3 and *n*-6 HUFA, respectively, whereas the total HUFA content was 1.66%. These values are very close to the 1% *n*-3 HUFA (w/w) level that should satisfy the requirements of penaeids as previously suggested

by Kanazawa (in D'Abramo, 1991). Eicosapentaenoic (20:5*n*-3) and docosahexaenoic acids (22:6*n*-3) made up respectively 0.85 and 0.32% of the diet.

The *n*-3 HUFA content of the diet has a large impact on that of the shrimp. Increases in the *n*-3 HUFA content of *Artemia* were followed by increases in the concentration of the corresponding fatty acids in the postlarvae. Postlarvae showed a particularly well developed ability to incorporate 22:6*n*-3, the content of which was directly proportional to that in *Artemia*. The 20:5*n*-3/22:6*n*-3 ratio decreased with that in the *Artemia*. However, the lower 20:5*n*-3/22:6*n*-3 values observed in PL-15 shrimp previously fed *Artemia* containing a little more than two times more 20:5*n*-3 than 22:6*n*-3 suggests that the shrimp may eventually concentrate 22:6*n*-3 in preference to 20:5*n*-3.

The *n*-3/*n*-6 HUFA ratio in postlarvae also increased with that of their *Artemia* diet. However, a saturation level appeared to be reached when the *n*-3/*n*-6 HUFA ratio in *Artemia* was higher than 2. These values yielded a *n*-3/*n*-6 HUFA of 3–4 in tissues of *P. monodon* postlarvae. Thus it might be that the quantitative *n*-6 HUFA requirement of *P. monodon* postlarvae corresponds to about one-fourth of their need for *n*-3 HUFA.

We might conclude by saying that *P. monodon* postlarvae can grow well on an *Artemia* diet containing low amounts of *n*-3 HUFA. However, since their survival was highly affected by the *n*-3 HUFA content of the diet, the overall production outputs would only be optimal when the dietary *n*-3 HUFA supply ranges from 12 to 22 mg/g DW. Early postlarval stages fed *Artemia* with a lower *n*-3 HUFA content would have reduced resistance to stressful conditions, such as water quality fluctuations or, as our data suggest, attacks by pathogens. Providing the postlarvae with a high dietary supply of HUFA would considerably enhance their ability to sustain stress, and would eventually improve their survival. However, excessive dietary *n*-3 HUFA levels ($\geqslant$31.2 mg/g DW) may lead to detrimental effects on both the growth and survival of the postlarvae.

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